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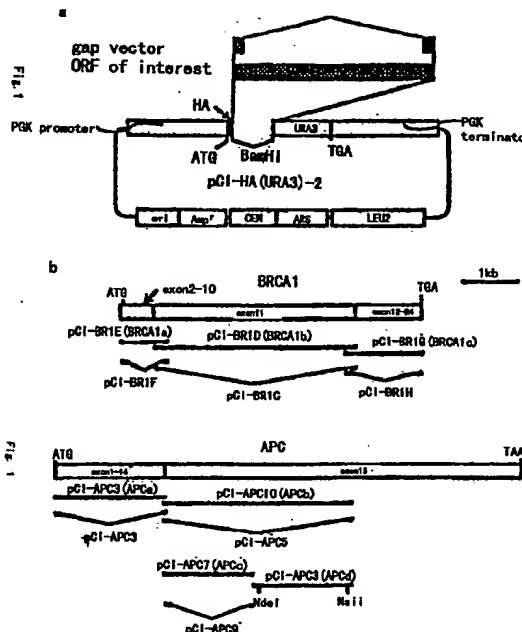
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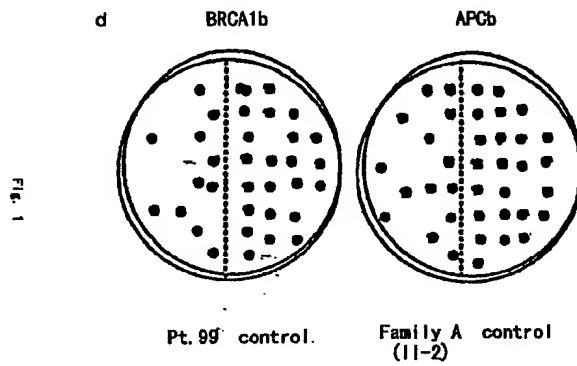
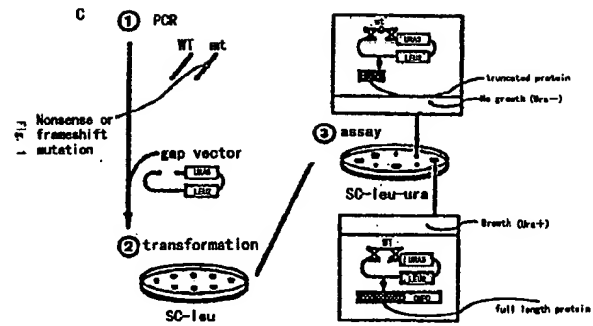
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(57) A method for detecting nonsense mutations and frameshift mutations which is simple and which may be applied even when the size of the test DNA is large is disclosed. In the method of the present invention, a test nucleic acid fragment is inserted into a site of a vector having a promoter, a translational initiation codon downstream of the promoter, a reporter gene which is a structural gene located downstream of the translational initiation codon, which is operably linked to the promoter and which encodes a polypeptide. A fusion polypeptide formed by ligating the N-terminal of the polypeptide to another polypeptide is detectable based on a function of the polypeptide encoded by the reporter gene. The site into which the test nucleic acid fragment is inserted is located downstream of the translational initiation codon and upstream of the reporter gene, and the test nucleic acid fragment is one which allows, when inserted, *in-frame* location of the reporter gene with respect to the translational initiation codon when the test nucleic acid fragment is normal type. The test nucleic acid fragment and the reporter gene downstream thereof in the resulting recombinant vector are then expressed in a host cell, and whether or not the fusion polypeptide having the function of the polypeptide encoded by the reporter gene is produced is determined.



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DescriptionTECHNICAL FIELD

5 The present invention relates to a method for detecting nonsense mutations and frameshift mutations.

BACKGROUND ART

10 If a structural gene has a nonsense mutation or a frameshift mutation therein, normal protein is not produced. That is, in case of a nonsense mutation, the amino acid sequence encoded by the region downstream of the mutated site is not produced at all, so that a protein shorter than the normal protein is produced. In case of a frameshift mutation, the amino acid sequence encoded by the region downstream of the mutated site is completely different from that of the normal amino acid sequence. Therefore, it is thought that, in general, existence of a nonsense mutation or a frameshift mutation in a structural gene results in a disease. Thus, it is clinically important to detect nonsense mutations and frameshift mutations in structural genes.

15 A method for detecting nonsense mutations or frameshift mutations in structural genes is, the method for measuring the activities of the proteins encoded by the structural genes. However, measurement of protein activities often requires complicated operations and there are a number of normal proteins which do not have measurable activities. Nonsense mutations and frameshift mutations can also be detected by sequencing the entire test gene. However, this method is complicated and laborious especially when the size of the test gene is large. Mutations of DNAs can also be sensitively detected by PCR-SSCP (single-stranded conformation polymorphism). However, this method necessitates electrophoresis and it is impossible to distinguish nonsense mutations from other point mutations. Further, this method cannot be applied to large DNAs.

25 DISCLOSURE OF THE INVENTION

Accordingly, an object of the present invention is to provide a method for detecting nonsense mutations and frameshift mutations, which is simple, and which may be applied to large DNAs.

30 The present inventors intensively studied to discover that nonsense mutations and frameshift mutations may be detected by using as a reporter gene a structural gene encoding a polypeptide detectable based on a function thereof, inserting a test nucleic acid fragment into a site upstream of the reporter gene, which test nucleic acid fragment does not shift the open reading frame of the reporter gene when the test nucleic acid fragment is normal type, expressing the test nucleic acid fragment and the reporter gene downstream thereof, and by determining whether or not a fusion polypeptide having the function of the polypeptide encoded by the reporter gene is produced, thereby completing the present invention.

35 That is, the present invention provides a method for detecting nonsense mutations and frameshift mutations comprising the steps of inserting a test nucleic acid fragment into a site of a vector having a promoter, a translational initiation codon downstream of the promoter, a reporter gene which is a structural gene located downstream of the translational initiation codon, which is operably linked to the promoter, which encodes a polypeptide, a fusion polypeptide formed by ligating the N-terminal of the polypeptide to another polypeptide being detectable based on a function of the polypeptide encoded by the reporter gene, the site into which the test nucleic acid fragment is inserted being located downstream of the translational initiation codon and upstream of the reporter gene, the test nucleic acid fragment being one which allows, when inserted, *in-frame* location of the reporter gene with respect to the translational initiation codon when the test nucleic acid is normal type; expressing the test nucleic acid fragment and the reporter gene downstream thereof in the resulting recombinant vector in a host cell, and determining whether the fusion polypeptide having the function of the polypeptide encoded by the reporter gene is produced or not.

40 By the present invention, a method by which nonsense mutations and frameshift mutations alone may be specifically detected by simple operations was provided. By the present invention, since detection may be made without operations such as electrophoresis, the operations are very simple. Further, by using a eukaryotic cell such as yeast cell as a host, large test nucleic acid fragment up to about 3.5 kb may be examined, so that the number of fragments when a gene is divided may be reduced. Further, since mutations close to PCR-primer flanking sequences may be detected, the sizes of the overlapping regions between the divided fragments may be reduced. Still further, mutations of heterozygous genes may also be detected.

55 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a drawing for explaining the structures of the vectors used in the methods of the Examples of the present invention as well as the regions of the test DNA fragments, operations and results.

Fig. 2 shows the pedigrees of 6 families which were examined for their APC genes by the method of Example 2 according to the present invention as well as the sizes of the bands observed in electrophoresis analyzing the vicinity of the mutated sites.

Fig. 3 shows arrangements of the regions in the BRCA2 gene, which were used as the test nucleic acid fragments in Example 3 of the present invention, as well as the restriction sites.

Fig. 4 shows the structures of the inserts in the recombinant vectors pCI-MS19 and pCI-MS20, which were used as test nucleic acid fragments in Example 4 of the present invention.

BEST MODE FOR CARRYING OUT THE INVENTION

In the method of the present invention, a vector is used. The vector has a structural gene used as a reporter gene. This reporter gene encodes a polypeptide detectable based on a function thereof, and the fusion polypeptide formed by ligating the N-terminal of the polypeptide to another polypeptide is also detectable based on the function of the polypeptide encoded by the reporter gene. Examples of such a reporter gene include genes which convert auxotroph to prototroph, genes giving drug resistance, genes encoding enzymes which carry out detectable enzyme reactions and genes which convert temperature-sensitive or pH-sensitive cells to resistant cells, but the reporter gene is not restricted to these genes. Among these, genes which convert auxotroph to prototroph, and genes giving drug resistance are preferred because detection may be carried out by simply culturing transformants on a prescribed medium. In the Examples described below, the region of codon 5 and downstream thereof of URA3 gene (Alani E. et al., Genetics 117,5-12(1987)) encoding yeast orotidine-5'-phosphate (OMP) decarboxylase is used as the reporter gene. Cells expressing this gene grow on a medium which does not contain uracil while the cells which do not express this gene cannot grow on a medium which does not contain uracil. Therefore, by employing a host auxotrophic to uracil and by culturing the transformant on a medium which does not contain uracil, whether or not the transformant expresses URA3 gene may easily be known. Further, cells expressing URA3 gene may easily be negatively selected using 5FOA (5-fluoro-orotic acid) (i.e., Ura⁻ is 5FOA-resistant and Ura⁺ is 5FOA-sensitive). Preferred examples of the reporter gene other than URA3 gene include GFP (green fluorescent protein) gene (Chalfie, M. et al., Science 236, 802-805, 1994), ADE2 gene (originated from yeast, Stotz, A. et al., Gene 95, 91-98, 1990), and CAN1 gene and CYH2 gene (both are described in Nature 387, 29, May, 1997), but the reporter gene is not restricted to these genes.

A promoter which controls the reporter gene is located upstream of the reporter gene. This promoter may be any promoter which can express the reporter gene downstream thereof in the host cell used, and known promoters may be employed. In the Examples described below, the promoter of 3-phosphoglycerate kinase (PKG) gene is used. Needless to say, however, the promoter is not restricted to this promoter. Preferred examples of the promoter other than the promoter of PKG gene include the promoters of ADH1 gene (budding yeast, Ammever, G., Methods in Enzymology, vol. 101, p.192), GAL1-GAL1D gene and of PHO5 gene (both are described in Broach, J.R. et al., "Experimental Manipulation of Gene Expression" Academic Press 1983), but the promoters are not restricted to these promoters.

A translational initiation codon is located downstream of the promoter and upstream of the reporter gene. The reporter gene is translated using this translation initiation codon as its translational initiation codon. Although the translational initiation codon may exist individually, it may be contained in a second structural gene located upstream of the reporter gene. In this case, whether or not the DNA region controlled by the promoter was correctly expressed can be known by detecting the polypeptide encoded by the second structural gene by an appropriate method such as immunoassay, Western blotting or the like. This is because that when expression is correctly carried out, at least the second structural gene is expressed even when the test nucleic acid fragment contains a nonsense mutation since the test nucleic acid fragment is inserted into a site downstream of the second structural gene as hereinbelow described. The second structural gene may be any structural gene. Although hemagglutinin-(HA) gene is used in the Examples described below, the second structural gene is, needless to say, not restricted to this gene.

Although the vector used in the method of the present invention may be a vector for bacteria such as *Escherichia coli* and *Bacillus subtilis*, a vector for *eukaryotic cells*, especially for yeasts, into which a large gene fragment can be inserted, is preferred.

Since the vector must be replicated in the host cell, the vector has a replication origin which enables its replication in the host cell. The vector may preferably have a terminator sequence downstream of the reporter gene. Further, the vector may preferably have a selection marker such as a drug resistant gene or a gene which converts auxotroph to prototroph (in the Examples described below, LEU2 gene which converts leucine auxotroph to leucine prototroph is used). Further, in case of a vector for yeasts, the vector may preferably have CEN gene or ARS gene for stabilizing replication in a small copy number.

In the method of the present invention, a test nucleic acid fragment is inserted into a site downstream of the translational initiation codon and upstream of the reporter gene. In cases where a second structural gene exists, the test nucleic acid fragment is inserted between the second structural gene and the reporter gene. The test nucleic acid fragment may be inserted into a restriction site existing upstream of the reporter gene by a conventional method. Thus, a

restriction site must exist upstream of the reporter gene. In cases where the vector used in the present invention is constructed by inserting the reporter gene and the second structural gene into an expression vector, a restriction site necessary for the insertion of the test nucleic acid fragment exist without any specific operation since the reporter gene and the second structural gene are inserted utilizing a restriction site. However, the restriction site needed for the insertion of the test nucleic acid fragment may easily be created by a conventional method.

The test nucleic acid fragment has a size that the open reading frame of the reporter gene is *in-frame* with the translational initiation codon when the test nucleic acid fragment is normal type. That is, when the open reading frame of the reporter gene is *in-frame* with the translational initiation codon before insertion of the test nucleic acid fragment, the number of the nucleotides in the test nucleic acid fragment is a multiple of 3 when the test nucleic acid fragment is normal type. This is because that if the number of the nucleotides in the test nucleic acid fragment to be inserted is a multiple of 3, frameshift does not occur. It should be noted, however, that the method of the present invention can be applied as long as the open reading frame of the reporter gene is *in-frame* with the translational initiation codon after a normal type DNA fragment is inserted. Therefore, the method of the present invention may be applied to those test nucleic acid fragments whose numbers of nucleotides are not a multiple of 3 when they are normal type. In this case, the open reading frame of the reporter gene and the translational initiation codon are preliminarily shifted before the insertion of the test nucleic acid fragment such that the open reading frame of the reporter gene and the translational initiation codon becomes *in-frame* after a normal type test nucleic acid is inserted.

The test nucleic acid fragment is not restricted at all and the test nucleic acid fragment may be any nucleic acid fragment (DNA fragment or RNA fragment) which is desired to be checked for the existence of a nonsense mutation or frameshift mutation. Although BRCA1 gene and APC gene are checked in the Examples described below, the test nucleic acid is, needless to say, not restricted to these genes. BRCA1 gene is the gene which may cause familial breast cancer and ovarian cancer when the gene is inactivated (Miki Y. et al., Science 266, 66-71 (1994), abnormality of this gene is observed in 75% of breast cancer patients), and APC gene is a gene which may cause multiple adenomatous polyp when the gene is inactivated (Kinzler K.W. et al., Science 253, 661-5(1991), abnormality of this gene is observed in 93% of patients). Although it is not necessary to know the nucleotide sequence of the test nucleic acid fragment, the number of the nucleotides of the normal type of the test nucleic acid fragment (or at least the remainder when the number of the nucleotides of the test nucleic acid fragment is divided by 3) must be known since the open reading frame of the reporter gene must be *in-frame* with the translational initiation codon after the normal type test nucleic acid fragment is inserted. Therefore, as the test nucleic acid fragment, one obtained by amplifying a known gene or a part thereof by a nucleic acid amplification method such as PCR or one obtained by cutting out a fragment from a known gene or a part thereof by a restriction enzyme is usually used. It is preferred to prepare the test nucleic acid fragment by a nucleic acid amplification method such as PCR because a large number of fragment can be obtained so that sensitivity of the assay is increased.

After insertion of the test nucleic acid fragment, host cells are transformed with the obtained recombinant vector. The method for transformation *per se* is well-known in the art. Alternatively, host cells may be cotransformed with the test nucleic acid fragment and a gap vector cleaved at the restriction site into which the test nucleic acid fragment is to be inserted so that the desired recombinant vector having the inserted test nucleic acid fragment is constructed by homologous recombination in the host cells (see Examples below). Here, the gap vector is a vector before insertion of the test nucleic acid fragment, which has the both end regions of the test nucleic acid fragments ligated to the respective ends of the vector which ends are generated by cleavage of the vector at the restriction site into which the test nucleic acid fragment is to be inserted. By cotransforming the host with such a gap vector and the test nucleic acid fragment, since the both end regions of the test nucleic acid fragments and the regions ligated to the restriction site of the gap vector are homologous, homologous recombination occurs in the cells, so that the recombinant vector into which the test nucleic acid fragment is inserted is generated in the cells. As described in the Examples below, such a gap vector may be prepared by firstly constructing a recombinant vector into which the test nucleic acid fragment is inserted and by amplifying the region of the recombinant vector other than the test nucleic acid fragment except for the both end regions thereof. By preliminarily preparing such a gap vector in a large amount, since the test may be carried out only by the cotransformation and culturing, the test is simple, so that clinical tests may be carried out efficiently on a number of samples, which is preferred. Further, the method employing the gap vector is preferred to the method in which a ligation mixture containing the vector into which the test nucleic acid fragment has been inserted is used as it is for the transformation also because the background of Ura^r is lower. The transformants may be selected depending on the selection marker.

The obtained transformants are then cultured so as to express the translational initiation codon (and the second structural gene in cases where it is contained), the inserted test nucleic acid fragment downstream thereof and the reporter gene, which are operably linked to the promoter.

As mentioned above, the test nucleic acid fragment has, if it is normal type, the size with which the open reading frame of the reporter gene is *in-frame* with the translational initiation codon when the test nucleic acid fragment is inserted. Therefore, in cases where the test nucleic acid fragment is normal type or has a mutation other than nonsense

mutation or frameshift mutation, a fusion polypeptide having the normal polypeptide encoded by the reporter gene is produced. On the other hand, in cases where the test nucleic acid has a nonsense mutation, the region downstream of the mutated site is not expressed, so that the reporter gene is not expressed at all. In cases where the test nucleic acid fragment has a frameshift mutation, since frameshift occurs in the region downstream of the mutated site, the polypeptide encoded by the reporter gene has an amino acid sequence totally different from that of the normal polypeptide, so that it does not have the function which the normal type polypeptide has. Therefore, by determining whether or not the expressed polypeptide has the function of the normal polypeptide encoded by the reporter gene, it can be determined whether or not the test DNA fragment has a nonsense or frameshift mutation.

Whether or not the expressed polypeptide has the function which the normal polypeptide encoded by the reporter gene has may be determined by an appropriate method depending on the nature of the reporter gene. That is, in cases where the reporter gene is one which converts auxotroph to prototroph, it may be determined by employing an auxotrophic strain as the host and culturing the transformants on a medium which does not contain the required nutrition. In cases where the reporter gene is a drug resistant gene, it may be determined by employing a host which is sensitive to the drug and by culturing the transformants on the medium containing the drug. In cases where the reporter gene encodes an enzyme which performs a detectable enzyme reaction, it may be determined by adding the substrate of the enzyme so as to allow the enzyme reaction. In cases where the reporter gene converts temperature-sensitive or pH-sensitive cells to resistant cells, it may be determined by employing a sensitive strain as the host, and by culturing the transformants under a temperature or pH at which the sensitive strain cannot grow. In cases where the reporter gene encodes a fluorescent protein, it may be determined by exciting the transformants with a light having the specific wavelength and by measuring the change of the characteristics of the fluorescent wavelength.

The present invention will now be described by way of examples thereof. It should be noted that the present invention is not restricted to the examples below.

Example 1 Detection of Nonsense Mutation or Frameshift Mutation in BRCA1 Gene

(1) Construction of Gap Vector

A fragment spanning nucleotide-number 423 to 1239nt of a plasmid pRS316 (Sikorski, R.S. et al., Genetics 122, 19-27 (1989), GenBank U03442, obtained from Robert S. Sikorski, Johns Hopkins University), which contains URA3 coding region from codon 5 to the natural termination codon, was amplified by PCR using a set of primers containing a BamHI site or a BglII site at the 5' end. The amplified BamHI/BglII fragment was inserted *in-frame* into a BamHI site of a plasmid pRSPGK (Ishioaka et al., Oncogene 10, 1485-92(1995), obtained from Chikashi ISHIOKA, Institute of Development, Aging and Cancer, Tohoku University) to produce a plasmid pCI-HA(URA3). This vector was digested by NsiI and PstI and was self-ligated to produce pCI-HA(URA3)-2 (Fig. 1, a).

On the other hand, genomic DNAs and/or total RNAs were isolated from lymphocytes immortalized by EBV, from 9 women with early-onset breast cancer, and from lymphocytes from healthy donors as controls. The nucleotide sequences of the coding regions of BRCA1 gene of the patients and the healthy donors have been fully determined (FitzGerald, M.G. et al., New Engl. J. Med. 334, 143-9 (1996); GenBank U14680). Using a commercially available cDNA synthesis kit (First-Strand cDNA Synthesis kit (commercially available from Pharmacia), cDNAs were synthesized. Using the thus obtained genomic DNAs and cDNAs as templates, test DNA fragments were amplified by PCR. The amplified test DNA fragments were the fragment spanning 96-908nt (BRCA1a), the fragment spanning 789-4214nt (BRCA1b) and the fragment spanning 4089-5708nt (BRCA1c) (see Fig. 1, b). BRCA1a and BRCA1c were produced by amplification using the cDNA as the template and BRCA1b was produced by amplification using each of the cDNA and genomic DNA as the template. The nucleotide sequences of the primers used for the amplification of BRCA1a were 5'-GAAAGTTCATTGGAACAGAAAGAA-3' and 5'-ACCCTGATACTTTTCTGGATG-3'. The nucleotide sequences of the primers used for the amplification of BRCA1b were 5'-CCCAGATCTGCTGCTTGTGAATTTTCTGAG-3' and 5'-CCCA-GATCTTAAGTTTGAATCCATGCTTTG-3'. The nucleotide sequences of the primers used for the amplification of BRCA1c were 5'-ATGAGGCATCAGTCTGAAAGC-3' and 5'-GTAGTGGCTGTGGGGGATCT-3'. PCR was performed using a kit commercially available from Takara Shuzo, which was performed by firstly carrying out an initial denaturing step at 94°C for 4 minutes, then repeating 30 times the cycle of denaturing step at 94°C for 1 minute, annealing step at 60°C for 1 minute and extension step at 72°C for 3.5 minutes, and by finally carrying out an extension step at 72°C for 4 minutes.

Amplified BRCA1a, BRCA1b and BRCA1c fragments originated from a healthy donor were respectively inserted into the BamHI site of pCI-HA(URA3)-2 to produce plasmids pCI-BR1E, pCI-BR1D and pCI-BR1G, respectively (see Fig. 1, b). *Saccharomyces cerevisiae* YPH499 (*infra*) was transformed with each of these recombinant vectors. As a result, all of the transformants were URA⁺ (transformants which can grow on a medium which does not contain uracil).

Using the obtained recombinant vectors as templates, gap vectors were prepared by PCR (Fig. 1, b). Gap vector pCI-BR1F is one prepared by changing the 183-827nt of the insert in the plasmid pCI-BR1E to a unique (i.e., only one

site exists in the vector) BglII site. Gap vector pCI-BR1C is one prepared by changing the 888-4111nt of the insert in the plasmid pCI-BR1D to a unique StuI/BamHI/SmaI site. Gap vector pCI-BR1H is one prepared by changing the 4215-5609nt of the insert in the plasmid pCI-BR1G to a unique BglII site. The nucleotide sequences of these plasmids are described in GenBank U14680. PCR was performed using the above-described full-length recombinant vector containing the insert fragment as the template, using the kit commercially available from Takara Shuzo. The nucleotide sequences of the primers used for preparing the gap vector pCI-BR1F were 5'-GAAGATCTGATTTTCTGCATAGCAT-TAATGAC-3' and 5'-GAAGATCTGAACATCATCAACCCAGTAATAATG-3'. The nucleotide sequences of the primers used for preparing the gap vector pCI-BR1C were 5'-CCCGGATCCCGGGAGTTGGTCTGAGTGACA-3' and 5'-CCCGGATCCAGGCCTCTCAGCTGCACGCTTC-3'. The nucleotide sequences of the primers used for preparing the gap vector pCI-BR1H were 5'-GAAGATCTCCTGTGGTGACCCGAGAGTGGGTG-3' and 5'-GAAGATCTATTATTTCTTCCAAGCCCGTTCC-3'. PCR was performed by firstly carrying out an initial denaturing step at 94°C for 2 minutes, then repeating 30 times the cycle of denaturing step at 94°C for 20 seconds, annealing plus extension step at 68°C for 10 minutes, and by finally carrying out an extension step at 68°C for 4 minutes.

(2) Production of Transformants

As the host cell, yeast *Saccharomyces cerevisiae* YPH499 (commercially available from Stratagene) which is auxotrophic to leucine and uracil was used. Competent yeast cells were prepared by treating the cells cultured in YPD liquid medium with lithium acetate (LiOAc) (Ishioka C. et al., Nature Genet. 5, 124-129(1993)). The obtained competent yeast cells were stored at -80°C in the presence of 5% DMSO until use. Frozen competent yeast cells retain high transformation efficiency at least for three months. Cotransformation was carried out by the known LiOAc method (the method described in Ito H., J. Bacteriol. 153, 163-168(1983) was modified as described in Ishioka H. et al., 1998 (*supra*)) with about 200 ng of the above-described each amplified DNA fragment (unpurified) and about 30 ng of the above-described each gap vector. For the tests of BRCA1a, BRCA1b and BRCA1c fragments, the gap vectors pCI-BR1F (BglII digest), pCI-BR1C (BamHI/SmaI digest) and pCI-BR1H (BglII digest) were used, respectively.

Transformants were selected on a synthetic complete medium which did not contain leucine. Twenty five colonies were selected from each group of the transformants and the transformants were cultured on a synthetic complete medium which did not contain leucine and uracil, thereby analyzing the uracil auxotrophy. In cases where more than 85% of transformants were URA⁺, the test DNA fragment was judged not to contain a nonsense or frameshift mutation. In cases where all transformants were URA⁺, the test DNA fragment was judged to contain a nonsense or frameshift mutation. In cases where the ratio of URA⁺ was small (usually 40 to 50%), the DNA fragment was judged to be a heterozygote having one which contained a nonsense or frameshift mutation and one which did not contain a nonsense or frameshift mutation.

(3) Results

The results are shown in Table 1 below. As shown in Table 1, the results obtained by the above-described method were completely coincident with the results of the analysis of the nucleotide sequences of the test DNA fragments. Thus, it was confirmed that nonsense mutation or frameshift mutation can be detected by the method of the present invention. The results of the patient Pt.99 (see Table 1) are shown in the left side in Fig. 1, d. The right half of the petri dish shows the results of the control in which the DNA fragment from a healthy donor was inserted and the left half shows the results of the patient Pt.99.

Table 1

Patient ^{*1}	Ratio of Ura ⁺ Colonies (%) ^{*2}				Mutation ^{*3}	
	BRCA1a	BRCA1b ^{*4}	BRCA1b ^{*5}	BRCA1c	Sequence	Location
Pt. 43	92	ND	88	92	Wild Type	codon 327 (exon 11)
Pt. 79	ND	92	ND	ND	Wild Type	
Pt. 84	92	91	92	96	Wild Type	
Pt. 99	96	<u>44</u>	<u>48</u>	88	2bp deletion (frameshift)	
Pt. 103	88	ND	88	96	Wild Type	
Pt. 118	88	94	94	88	Wild Type	codon 23 (exon 2)
Pt. 231	<u>44</u>	ND	88	92	2bp deletion (frameshift)	
Pt. 253	<u>48</u>	ND	88	100	2bp deletion (frameshift)	
Pt. 364	96	ND	<u>44</u>	92	CGA to TGA (nonsense)	
ND: not determined						

*1, *3: All patients are women with breast cancer before the age 30 and have been characterized for BRCA1 mutations previously.

*2: Underscored number indicates heterozygote having a gene with a nonsense or frameshift mutation and a normal gene.

*4: Derived from genomic DNA of patient lymphocytes.

*5: Derived from first-strand cDNA of patient lymphocytes.

Example 2 Detection of Nonsense Mutation or Frameshift Mutation in APC Gene

By the method similar to Example 1, N-terminal side (about 61% of the entire gene) of APC genes of 6 families of patients suffering from familial multiple adenomatous polyp was analyzed. It is known that about 93% of the patients of the cancer have mutations of APC gene (Nakamura Y. et al., New Strategies for Treatment of Hereditary Colorectal Cancer, Ed. S. Baba et al., 1996, pp.93-98). Most of the mutations are located in the above-mentioned N-terminal side.

Test DNA fragments were prepared by using genomic DNA or cDNA from fresh lymphocytes from patients and a healthy donor as the templates. The amplified test DNA fragments were 19-1977nt (APCa), 1978-5256nt (APCb), 1978-3570nt (APCc) and 3571-5256nt (APCd) of APC gene (see Fig. 1, b). The nucleotide sequences of the primers used for the amplification of APCa fragment were 5'-ATGGCTGCAGCTTCATATGAT-3' and 5'-CTGTGGTCCTCATTGTAGC-3'. The nucleotide sequences of the primers used for the amplification of APCb fragment were 5'-CAAATCCTAAGAGA-GAACAAC-3' and 5'-GTCCATTATCTTTTTCACACG-3'. The nucleotide sequences of the primers used for the amplification of APCc fragment were 5'-CAAATCCTAAGAGAGAACA-3' and 5'-GGCATATTTAACTATAATC-3'. The nucleotide sequences of the primers used for the amplification of APCd fragment were 5'-ACAGATATTCCTTCAT-CACAG-3' and 5'-GTCCATTATCTTTTTCACACG-3'. The PCR was performed under the same conditions as in Example 1.

Amplified APCa, APCb and APCc fragments originated from healthy donor were respectively inserted into the BamHI site of pCI-HA(URA3)-2 to produce plasmids pCI-APC6, pCI-APC10 and pCI-APC7, respectively (see Fig. 1, b). *Saccharomyces cerevisiae* YPH499 was transformed with each of these recombinant vectors. As a result, all of the transformants were URA⁺.

Using the obtained recombinant vectors as templates, gap vectors were produced by PCR (Fig. 1, b). Gap vector pCI-APC8 is one prepared by changing the 109-1899nt of the insert in the plasmid pCI-APC6 to a unique BglII site. Gap vector pCI-APC5 is one prepared by changing the 2054-5201nt of the insert in the plasmid pCI-APC10 to a unique NsiI site. Gap vector pCI-APC9 is one prepared by changing the 2086-3489nt of the insert in the plasmid pCI-APC7 to a unique BglII site. The nucleotide sequences of these plasmids are described in GenBank M74088. The nucleotide sequences of the primers used for preparing the gap vector pCI-APC8 were 5'-CGAAGATCTATTATCTTCTAGCTCTT-GTGAAG-3' and 5'-CGAAGATCTACTTTAGCCATTATTGAAGTGA-3'. The nucleotide sequences of the primers used for preparing the gap vector pCI-APC9 were 5'-CGAAGATCTTGCTGAGAGATTCCACAAAGTTCC-3' and 5'-CGAAGATCTAGACCAACAAATTATAGCATAAAATAT-3'. PCR was performed using the full-length recombinant vectors each of which contains the respective insert under the same conditions as in Example 1. Gap vector pCI-APC5 was prepared by cleaving pCI-APC10 at the two NsiI sites therein by NsiI.

Yeast *Saccharomyces cerevisiae* YPH499 was cotransformed by the same method as in Example 1 with the respective gap vector cleaved at its above-mentioned unique restriction site and the previously prepared test DNA fragment. Transformants were selected and existence of a nonsense or frameshift mutation was checked.

5 The results are shown in Tables 2 and 3. Pedigrees of the tested 6 families are shown in Fig. 2. In Fig. 2, the individuals indicated by solid symbols are those in whom a nonsense or frameshift mutation was detected. As shown in Tables 2 and 3, the results obtained by the above-described method were completely coincident with the results of the analysis of the nucleotide sequences of the test DNA fragments. Thus, it was confirmed that nonsense mutation or frameshift mutation can be detected by the method of the present invention. The results of the patient II-2 (see Table 2) in Family A are schematically shown in the right side in Fig. 1, d. The right half of the petri dish shows the results of the control in which the DNA fragment from the healthy donor was inserted and the left half shows the results of the patient
10 II-2 in Family A.

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Table 2

FAP Family ^{*1}	Ratio of Ura ⁺ Colony (%) ^{*2}			Mutation			
	APCb	APCc	APCd	Location	Sequence, ^{*3}	Consequence	Name
Family A	100	92	ND	codon 929-930(Exon 15)	CATA <u>CA</u> →CA	Frameshift	2805del14
	38	48	92				
	56	48	ND				
Family B	50	44	100	codon 938 (Exon15)	ACTAAG→ACTTAG	Frameshift	2831inst
	96	92	ND				
	88	100	ND				
Family C	ND	ND	100	codon 1249-1250 (Exon 15)	TGCAAA→TGA	Nonsense	3765del2
	ND	ND	100				
	ND	ND	96				
	40	92	56				
	ND	ND	92				
	ND	ND	40				
	ND	ND	48				
	ND	ND	52				
	ND	ND	52				

Table 3

FAP Family*1	Mutation				Name
	APCb	APCc	APCd	Sequence*3	
Family D					
I-1	<u>60</u>	92	<u>56</u>	GAAAAGATT→GATT	3945del5
II-1	ND	ND	92		
II-2	ND	ND	96		
II-3	ND	ND	88		
Family E					
I-1	<u>44</u>	96	<u>48</u>	GAAAAGATT→GATT	3945del5
II-1	ND	ND	92		
II-2	ND	ND	<u>48</u>		
II-3	ND	ND	92		
Family F					
I-1	<u>57</u>	92	<u>48</u>	GAA→GA	3983delA
II-1	ND	ND	92		

ND: not determined

*1: All individuals in the 6 families of familial multiple adenomatous polyp are Japanese.

*2: Underscored number indicates heterozygote having a gene with a nonsense or frameshift mutation and a normal gene.

*3: Underscored nucleotides were deleted or inserted.

Each region around the mutated site was amplified by PCR. The PCR product was electrophoresed in 16% polyacrylamide gel and was visualized by ethidium bromide staining and analyzed. The size of each band obtained for each

family is shown below each family in the pedigree shown in Fig. 2. The results of each family will now be described.

Family A

5 Amplification of APC fragment spanning 2761-2877nt (GenBank M74088) generated an abnormally short (103 bp) fragment as well as a normal-sized (107 bp) fragment in affected siblings, II-2 and II-3 because of heterozygous 4 bp deletion at 2805nt (2805del4). Only 107 bp fragment was observed in II-1.

Family B

10 The identical PCR product with Family A was digested by AflII. In addition to a normal-sized (107 bp) fragment, a 71 bp fragment was observed in the proband, I-1, because insertion of T at 2831nt (2831insT) generates an AflII site, CTTAAG. This additional band was not found in II-1 and II-2.

Family C

15 Amplification of APC fragment spanning 3744-3827nt generated an abnormally short 82 bp fragment as well as a normal-sized 84 bp fragment in the proband, I-7 and three affected members (II-1, II-2 and II-3) because of heterozygous 2 bp deletion at 3765nt (3765del2). The additional band was not observed in other unaffected members.

Families D and E

20 Amplification of APC fragment spanning 3881-3964nt generated an abnormally short (79 bp) fragment as well as a normal-sized (84 bp) fragment in the probands, I-1 (both Families D and E) and an affected member, II-2 (Family E), because of heterozygous 5 bp deletion at 3945nt (3945del5). Only normal-sized product was observed in II-1 (both Families D and E), II-2 (Family D) and II-3 (both Families D and E).

Family F

30 The APC fragment spanning 3881-4004nt was amplified using a mismatch primer 5'-TGCTGTGACACTGCT-GGAGC-3' (the underscored G is the mismatch nucleotide) to generate T to C change at 3986nt and was digested by SacI. In addition to a normal-sized (124 bp) fragment, a 107 bp fragment was observed in the proband, I-1, because 1 bp deletion at 3983nt (3983delA) combined with the T to C change generates a SacI, GAGCTC. The 107 bp band was not observed in II-1.

35 Example 3 Detection of Nonsense Mutation or Frameshift Mutation in BRCA2 Gene

(1) Construction of BRCA2 Gap Vector

40 A fragment spanning 423-1239nt of a plasmid pRS316 (Sikorski, R.S. et al., Genetics 122, 19-27 (1989), GenBank U03442, obtained from Robert S. Sikorski, Johns Hopkins University), which contains URA3 coding region from codon 5 to the natural termination codon, was amplified by PCR using a set of primers containing a BamHI site or a BglII site at the 5' end. The amplified BamHI/BglII fragment was inserted *in-frame* into a BamHI site of a plasmid pRSPGK (Ishioaka et al., Oncogene 10, 1485-92(1995), obtained from Chikashi ISHIOKA, Institute of Development, Aging and Cancer, Tohoku University) to produce a plasmid pCI-HA(URA3). This vector was digested by NsiI and PstI and was self-ligated to produce pCI-HA(URA3)-2 (Fig. 1, a).

45 On the other hand, BR2a, BR2b, BR2c, BR2d and BR2e fragments originated from a healthy donor were amplified by PCR under the conditions described below. The arrangements of these fragments and restriction sites are shown in Fig. 3. The amplified DNA fragments were 219-2358nt (BR2a), 2149-4815nt (BR2b), 4639-6708nt (BR2c), 6493-8439nt (BR2d) and 8251-10476nt (BR2e) of BRCA2 gene. The nucleotide sequences of the primers used for the amplification of BR2a fragment were 5'-GGAAGATCTATGCCTATTGGATCCAAAGAGAG-3' and 5'-GGAAGATCTTGACA-GAATCAGCTTCTGGGG-3'. The nucleotide sequences of the primers used for the amplification of BR2b fragment were 5'-CGGGATCCTCTTCTGTGAAAAGAAGCTGTTCAC-3' and 5'-CGGGATCCCCCGCTAGCTGTATGAAAACCC-3'. The nucleotide sequences of the primers used for the amplification of BR2c fragment were 5'-CGGGATCCAGAAA-GAACAAAATGGACATTCTAAG-3' and 5'-CGGGATCCTTGTTGAAATTGAGAGAGATATGGAG-3'. The nucleotide sequences of the primers used for the amplification of BR2d fragment were 5'-GGAAGATCTGAGCATAGTCTTCACATAT-TCACCTAC-3' and 5'-GGAAGATCTTAAGAGGGGAGGATCTAACTGG-3'. The nucleotide sequences of the primers used for the amplification of BR2e fragment were 5'-CGGGATCCGATAGAAGCAGAAGATCGGCTATAA-3' and 5'-

CGGGATCCGATATATTTTGTAGTTGTAATTGTGTCCTG-3'. The PCR for BR2a was performed by firstly carrying out an initial denaturing step at 94°C for 4 minutes, then repeating 35 times the cycle of denaturing step at 94°C for 30 seconds and annealing + extension step at 68°C for 3 minutes (the annealing + extension step is prolonged for 4 seconds per cycle), and by finally carrying out an extension step at 72°C for 5 minutes. The PCR for BR2b and BR2c was performed by firstly carrying out an initial denaturing step at 94°C for 4 minutes, then repeating 27 times the cycle of denaturing step at 94°C for 30 seconds, annealing step at 60°C for 15 seconds and extension step at 72°C for 3 minutes + DT 4 seconds, and by finally carrying out an extension step at 72°C for 5 minutes. The PCR for BR2d was performed by firstly carrying out an initial denaturing step at 94°C for 4 minutes, then repeating 35 times the cycle of denaturing step at 94°C for 30 seconds, annealing step at 60°C for 15 seconds and extension step at 72°C for 3 minutes + DT 4 seconds, and by finally carrying out an extension step at 72°C for 5 minutes. The PCR for BR2e was performed by firstly carrying out an initial denaturing step at 94°C for 2 minutes, then repeating 35 times the cycle of denaturing step at 94°C for 30 seconds, annealing step at 58°C for 30 seconds and extension step at 72°C for 3 minutes + DT 4 seconds, and by finally carrying out an extension step at 72°C for 5 minutes.

Amplified BR2a, BR2b, BR2c, BR2d and BR2e fragments originated from a healthy donor were respectively inserted into the BamHI site of pCI-HA(URA3)-2 to produce plasmids pBR2a, pBR2b, pBR2c, pBR2d and pBR2e. *Saccharomyces cerevisiae* YPH499 was transformed with each of these recombinant vectors. As a result, all of the transformants were URA⁺.

A gap vector of pBR2a was prepared by digesting the recombinant vector pBR2a by restriction enzymes PstI and XbaI so as to remove the central region, 365-2239nt, of the insert. A gap vector of pBR2b was prepared by digesting the recombinant vector pBR2b by restriction enzymes XbaI and SpeI so as to remove the central region, 2239-4734nt, of the insert. A gap vector of pBR2c was prepared by digesting the recombinant vector pBR2c by restriction enzymes SpeI and PstI so as to remove the central region, 4734-6603nt, of the insert. A gap vector of pBR2d was prepared by digesting the recombinant vector pBR2d by restriction enzymes PstI and SpeI so as to remove the central region, 6603-8350nt, of the insert. A gap vector of pBR2e was prepared by digesting the recombinant vector pBR2e by restriction enzymes SpeI and BclI so as to remove the central region, 8350-10397nt, of the insert. The nucleotide sequences of these gap vectors are described in GenBank U43746.

(2) BRCA2 SC Assay

Entire regions of the open reading frames of 2 early-onset breast cancer patients and a healthy donor were analyzed.

Genomic DNAs and/or total RNAs were isolated from lymphocytes immortalized by EBV, from 2 women with early-onset breast cancer, and from lymphocytes from a healthy donor as a control. The nucleotide sequences of the coding regions of BRCA2 gene of the patients and the healthy donor are known. Using a commercially available cDNA synthesis kit (First-Strand cDNA Synthesis kit (commercially available from Pharmacia), cDNAs were synthesized. Using the thus obtained genomic DNAs and cDNAs as templates, test DNA fragments were amplified by PCR. The amplified test DNA fragments were the fragments spanning 219-2358nt (BR2a), 2149-4815nt (BR2b), 4639-6708nt (BR2c), 6493-8439nt (BR2d) and 8251-10476nt (BR2e), respectively. The nucleotide sequences of the primers used for the amplification of BR2a were 5'-ATGCCATTGGATCCAAAGAGAG-3' and 5'-TGACAGAATCAGCTTCTGCGG-3'. The nucleotide sequences of the primers used for the amplification of BR2b were 5'-TCTTCTGTGAAAAGAAGCTGTTTAC-3' and 5'-CCCGCTAGCTGTATGAAAACCC-3'. The nucleotide sequences of the primers used for the amplification of BR2c were 5'-AGAAAGAACAAAATGGACATTCTAAG-3' and 5'-TTGTTGAAATTGAGAGAGATATGGAG-3'. The nucleotide sequences of the primers used for the amplification of BR2d were 5'-TCTGAGCATAGTCTTCACTATTCACCTAC-3' and 5'-TCTTAAGAGGGGAGGATCTAACTGG-3'. The nucleotide sequences of the primers used for the amplification of BR2e were 5'-GATAGAAGCAGAAGATCGGCTATAA-3' and 5'-GATATATTTTGTAGTTGTAATTGTGTCCTG-3'. The PCR for BR2a was performed by firstly carrying out an initial denaturing step at 94°C for 4 minutes, then repeating 35 times the cycle of denaturing step at 94°C for 30 seconds and annealing + extension step at 68°C for 3 minutes + DT 4 seconds, and by finally carrying out an extension step at 72°C for 5 minutes. The PCR for BR2b and BR2c was performed by firstly carrying out an initial denaturing step at 94°C for 4 minutes, then repeating 27 times the cycle of denaturing step at 94°C for 30 seconds, annealing step at 60°C for 15 seconds and extension step at 72°C for 3 minutes + DT 4 seconds, and by finally carrying out an extension step at 72°C for 5 minutes. The PCR for BR2d was performed by firstly carrying out an initial denaturing step at 94°C for 4 minutes, then repeating 35 times the cycle of denaturing step at 94°C for 30 seconds, annealing step at 60°C for 15 seconds and extension step at 72°C for 3 minutes + DT 4 seconds, and by finally carrying out an extension step at 72°C for 5 minutes. The PCR for BR2e was performed by firstly carrying out an initial denaturing step at 94°C for 4 minutes, then repeating 35 times the cycle of denaturing step at 94°C for 30 seconds, annealing step at 58°C for 30 seconds and extension step at 72°C for 3 minutes + DT 4 seconds, and by finally carrying out an extension step at 72°C for 5 minutes.

(3) Detection

Yeast *Saccharomyces cerevisiae* YPH499 was cotransformed by the same method as in Examples 1 and 2 with the respective gap vector cleaved at the above-mentioned unique restriction site and the previously prepared test DNA fragment. Transformants was selected and existence of a nonsense or frameshift mutation was checked. Selection of the transformants was performed by using a uracil auxotroph as a host and 1) by culturing the transformants on a medium not containing uracil so as to positively select the transformants, and 2) by culturing the transformants on a medium containing 5FOA (5-fluoro-orotic acid) so as to negatively select the transformants.

(4) Results

The results are shown in Table 4. DNA sequence analysis was carried out for Patient 1 and Patient 2. In Patient 1, heterozygous 5146delTTTA (4 bp deletion) was detected in BR2c fragment. In Patient 2, heterozygous 6697delITC (2 bp deletion) was detected in BR2d fragment. Thus, the results obtained by the above-described method were completely coincident with the results of the analysis of the nucleotide sequences of the test DNA fragments. Thus, it was confirmed that nonsense mutation or frameshift mutation can be detected by the method of the present invention.

Table 4

	BR2a	BR2b	BR2c	BR2d	BR2e
Healthy Donor Control 1	88% 8%	92% 6%	94% 4%	90% 10%	84% 6%
Healthy Donor Control 2	92% 10%	92% 12%	92% 4%	86% 8%	86% 8%
Patient 1	86% 14%	92% 6%	40% 56%	84% 12%	90% 12%
Patient 2	88% 12%	100% 4%	92% 0%	38% 60%	88% 6%
	% Ura ⁺ / % SFOA ⁺				

Example 4 Detection of Nonsense Mutation or Frameshift Mutation in hMSH2 Gene

(1) Construction of hMSH2 Gap Vector

5 Full-length region of the open reading frame of hMSH2 (4-2805nt of GenBank U03911) originated from a healthy donor, which was amplified by PCR, was inserted into the BamHI site of pCI-HA(URA3)-2 to produce pCI-MS19. *Saccharomyces cerevisiae* YPH499 was transformed with the recombinant vector by a conventional method. All of the obtained transformants were URA⁺ and 5FOA-sensitive.

10 A gap vector pCI-MS20 was prepared by digesting the recombinant vector pCI-MS19 by restriction enzyme BglII so as to remove the central region, 97-2730nt, of the insert. The structures of the inserts in the recombinant vectors pCI-MS19 and pCI-MS20 are shown in Fig. 4.

(2) hMSH2 SC Assay

15 Entire regions of the open reading frame of hMSH2 genes of one patient suffering from hereditary non-polyposis colon cancer and of two healthy donors were analyzed.

Total RNAs were isolated from lymphocytes of one patient suffering from hereditary non-polyposis colon cancer and of two healthy donors were isolated. The nucleotide sequences of the coding regions of hMSH2 gene of the patients and the healthy donors are known. Using a commercially available cDNA synthesis kit (First-Strand cDNA Synthesis kit (commercially available from Pharmacia), cDNAs were synthesized. Using the thus obtained cDNAs as templates, test DNA fragments were amplified by PCR. The amplified test DNA fragments were the fragments spanning 4-2805nt of hMSH2 gene. The nucleotide sequences of the primers used for the amplification of fragments were 5'-ATGGCGGTGCAGCCGAAGGAGACGC-3' and 5'-CGTAGTAACTTTTATTCGTGAAATGATTTCATT-3'. The PCR was performed by firstly carrying out an initial denaturing step at 94°C for 4 minutes, then repeating 32 times the cycle of denaturing step at 94°C for 30 seconds, annealing-step at 60°C for 30 seconds and extension step at 72°C for 3 minutes, and by finally carrying out an extension step at 72°C for 5 minutes.

(3) Detection

30 Yeast *Saccharomyces cerevisiae* YPH499 was cotransformed by the same method as in Examples 1, 2 and 3 with the gap vector cleaved at its above-mentioned unique restriction site and the previously prepared test DNA fragment. Transformants were selected and existence of a nonsense or frameshift mutation was checked. Selection of the transformants was performed by using a uracil auxotroph as a host and 1) by culturing the transformants on a medium not containing uracil so as to positively select the transformants, and 2) by culturing the transformants on a medium containing 5FOA (5-fluoro-orotic acid) so as to negatively select the transformants.

(4) Results

40 The results are shown in Table 5. DNA sequence analysis was carried out for Patient 1. In Patient 1, heterozygous 2297delC (1 bp deletion) was detected in hMSH2 fragment. Thus, the results obtained by the above-described method were completely coincident with the results of the analysis of the nucleotide sequences of the test DNA fragments. Thus, it was confirmed that nonsense mutation or frameshift mutation can be detected by the method of the present invention.

Table 5

	% Ura ⁺	% 5FOA ⁺
Healthy Donor Control 1	86%	14%
Healthy Donor Control 2	92%	8%
Patient	40%	60%

SEQUENCE LISTING

5 SEQ ID NO: 1
SEQUENCE LENGTH: 24
SEQUENCE TYPE: nucleic acid
10 STRANDEDNESS: single
TOPOLOGY: linear
SEQUENCE DESCRIPTION
15 GAAAGTTCAT TGGAACAGAA AGAA 24

20 SEQ ID NO: 2
SEQUENCE LENGTH: 21
SEQUENCE TYPE: nucleic acid
25 STRANDEDNESS: single
TOPOLOGY: linear
SEQUENCE DESCRIPTION
30 ACCCTGATAC TTTTCTGGAT G 21

35 SEQ ID NO: 3
SEQUENCE LENGTH: 30
SEQUENCE TYPE: nucleic acid
40 STRANDEDNESS: single
TOPOLOGY: linear
SEQUENCE DESCRIPTION
45 CCCAGATCTG CTGCTTGTGA ATTTTCTGAG 30

50 SEQ ID NO: 4
SEQUENCE LENGTH: 30
55

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

CCCAGATCTT AAGTTTGAAT CCATGCTTTG

30

SEQ ID NO: 5

SEQUENCE LENGTH: 21

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

ATGAGGCATC AGTCTGAAAG C

21

SEQ ID NO: 6

SEQUENCE LENGTH: 20

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

GTAGTGGCTG TGGGGGATCT

20

SEQ ID NO: 7

SEQUENCE LENGTH: 32

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

GAAGATCTGA TTTTCTGCAT AGCATTAATG AC

32

5

SEQ ID NO: 8

10

SEQUENCE LENGTH: 33

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

15

TOPOLOGY: linear

SEQUENCE DESCRIPTION

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GAAGATCTGA ACATCATCAA CCCAGTAATA ATG

33

SEQ ID NO: 9

25

SEQUENCE LENGTH: 30

SEQUENCE TYPE: nucleic acid

30

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

35

CCCGGATCCC GGGAGTTGGT CTGAGTGACA

30

SEQ ID NO: 10

40

SEQUENCE LENGTH: 31

SEQUENCE TYPE: nucleic acid

45

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

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CCCGGATCCA GGCCTCTCAG CTGCACGCTT C

31

55

SEQ ID NO: 11

SEQUENCE LENGTH: 32

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

GAAGATCTCC TGTGGTGACC CGAGAGTGGG TG 32

SEQ ID NO: 12

SEQUENCE LENGTH: 32

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

GAAGATCTAT TATTTTCTTC CAAGCCCGTT CC 32

SEQ ID NO: 13

SEQUENCE LENGTH: 21

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

ATGGCTGCAG CTTTCATATGA T 21

SEQ ID NO: 14

SEQUENCE LENGTH: 20

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single
TOPOLOGY: linear
5 SEQUENCE DESCRIPTION
CTGTGGTCCT CATTTGTAGC 20
10
SEQ ID NO: 15
SEQUENCE LENGTH: 21
15 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear_
20 SEQUENCE DESCRIPTION
CAAATCCTAA GAGAGAACAA~C 21
25
SEQ ID NO: 16
SEQUENCE LENGTH: 21
30 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
35 TOPOLOGY: linear
SEQUENCE DESCRIPTION
GTCCATTATC TTTTTCACAC G 21
40
SEQ ID NO: 17
45 SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
50 TOPOLOGY: linear
SEQUENCE DESCRIPTION
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CAAATCCTAA GAGAGAACAA

20

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SEQ ID NO: 18

SEQUENCE LENGTH: 21

10

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

15

SEQUENCE DESCRIPTION

GGCATATTTT AAATAATAAT C

21

20

SEQ ID NO: 19

SEQUENCE LENGTH: 21

25

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

30

SEQUENCE DESCRIPTION

ACAGATATTC CTTCATCACA G

21

35

SEQ ID NO: 20

SEQUENCE LENGTH: 21

40

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

45

SEQUENCE DESCRIPTION

GTCCATTATC TTTTTCACAC G

21

50

SEQ ID NO: 21

55

SEQUENCE LENGTH: 33

SEQUENCE TYPE: nucleic acid

5 STRANDEDNESS: single

TOPOLOGY: linear

10 SEQUENCE DESCRIPTION

CGAAGATCTA TTATCTTCTA GCTCTTGTCG AAG 33

15 SEQ ID NO: 22

SEQUENCE LENGTH: 32

20 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

25 SEQUENCE DESCRIPTION

CGAAGATCTA CTTTAGCCAT TATTGAAGTG GA 32

30 SEQ ID NO: 23

SEQUENCE LENGTH: 33

35 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

40 SEQUENCE DESCRIPTION

CGAAGATCTT GCTGAGAGAT TCCACAAAGT TCC 33

45

SEQ ID NO: 24

SEQUENCE LENGTH: 36

50 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

55

TOPOLOGY: linear

SEQUENCE DESCRIPTION

5 CGAAGATCTA GACCAACAAA TTATAGCATA AAATAT 36

10 SEQ ID NO: 25

SEQUENCE LENGTH: 20

SEQUENCE TYPE: nucleic acid

15 STRANDEDNESS: single

TOPOLOGY: linear

20 SEQUENCE DESCRIPTION

TGCTGTGACA CTGCTGGAGC 20

25 SEQ ID NO: 26

SEQUENCE LENGTH: 32

SEQUENCE TYPE: nucleic acid

30 STRANDEDNESS: single

TOPOLOGY: linear

35 SEQUENCE DESCRIPTION

GGAAGATCTA TGCCTATTGG ATCCAAAGAG AG 32

40 SEQ ID NO: 27

SEQUENCE LENGTH: 30

SEQUENCE TYPE: nucleic acid

45 STRANDEDNESS: single

TOPOLOGY: linear

50 SEQUENCE DESCRIPTION

GGAAGATCTT GACAGAATCA GCTTCTGGGG 30

55

SEQ ID NO: 28

5 SEQUENCE LENGTH: 33

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

10 TOPOLOGY: linear

SEQUENCE DESCRIPTION

15 CGGGATCCTC TTCTGTGAAA AGAAGCTGTT CAC

33

SEQ ID NO: 29

20 SEQUENCE LENGTH: 30

SEQUENCE TYPE: nucleic acid

25 STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

30 CGGGATCCCC CGCTAGCTGT ATGAAAACCC

30

35 SEQ ID NO: 30

SEQUENCE LENGTH: 34

SEQUENCE TYPE: nucleic acid

40 STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

45 CGGGATCCAG AAAGAACAAA ATGGACATTC TAAG

34

50 SEQ ID NO: 31

SEQUENCE LENGTH: 34

55

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

5 TOPOLOGY: linear

SEQUENCE DESCRIPTION

10 CGGGATCCTT GTTGAAATG AGAGAGATAT GGAG 34

SEQ ID NO: 32

15 SEQUENCE LENGTH: 35

SEQUENCE TYPE: nucleic acid

20 STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

25 GGAAGATCTG AGCATAGTCT TCACTATTCA CCTAC 35

SEQ ID NO: 33

30 SEQUENCE LENGTH: 31

SEQUENCE TYPE: nucleic acid

35 STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

40 GGAAGATCTT AAGAGGGGAG GATCTAACTG G 31

SEQ ID NO: 34

45 SEQUENCE LENGTH: 33

SEQUENCE TYPE: nucleic acid

50 STRANDEDNESS: single

TOPOLOGY: linear

55

SEQUENCE DESCRIPTION

CGGGATCCGA TAGAAGCAGA AGATCGGCTA TAA 33

5

SEQ ID NO: 35

10 SEQUENCE LENGTH: 38

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

15 TOPOLOGY: linear

SEQUENCE DESCRIPTION

20 CGGGATCCGA TATATTTTFT AGTTGTAATT GTGTCCTG 38

SEQ ID NO: 36

25 SEQUENCE LENGTH: 23

SEQUENCE TYPE: nucleic acid

30 STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

35 ATGCCTATTG GATCCAAAGA GAG 23

40 SEQ ID NO: 37

SEQUENCE LENGTH: 21

SEQUENCE TYPE: nucleic acid

45 STRANDEDNESS: single

TOPOLOGY: linear

50 SEQUENCE DESCRIPTION

TGACAGAATC AGCTTCTGGG G 21

55

SEQ ID NO: 38

SEQUENCE LENGTH: 25

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

TCTTCTGTGA AAAGAAGCTG TTCAC

25

SEQ ID NO: 39

SEQUENCE LENGTH: 22

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

CCCGCTAGCT GTATGAAAAC CC

22

SEQ ID NO: 40

SEQUENCE LENGTH: 36

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

SEQUENCE DESCRIPTION

AGAAAGAACA AAATGGACAT TCTAAG

36

SEQ ID NO: 41

SEQUENCE LENGTH: 36

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

5 SEQUENCE DESCRIPTION

TTGTTGAAAT TGAGAGAGAT ATGGAG 36

10

SEQ ID NO: 42

SEQUENCE LENGTH: 29

15 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

20 SEQUENCE DESCRIPTION

TCTGAGCATA GTCTTCACTA TTCACCTAC 29

25

SEQ ID NO: 43

SEQUENCE LENGTH: 25

30 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

35 SEQUENCE DESCRIPTION

TCTTAAGAGG GGAGGATCTA ACTGG 25

40

SEQ ID NO: 44

SEQUENCE LENGTH: 25

45 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

50 SEQUENCE DESCRIPTION

55

GATAGAAGCA GAAGATCGGC TATAA

25

5

SEQ ID NO: 45

SEQUENCE LENGTH: 30

10

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

15

TOPOLOGY: linear

SEQUENCE DESCRIPTION

GATATATTTT TTAGTTGTAA TTGTGTCCTG

30

20

SEQ ID NO: 46

25

SEQUENCE LENGTH: 25

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

30

TOPOLOGY: linear

SEQUENCE DESCRIPTION

ATGGCGGTGC AGCCGAAGGA GACGC

25

35

SEQ ID NO: 47

40

SEQUENCE LENGTH: 33

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

45

TOPOLOGY: linear

SEQUENCE DESCRIPTION

CGTAGTAACT TTTATTCGTG AAATGATTTC ATT

33

50

55

Claims

1. A method for detecting nonsense mutations and frameshift mutations comprising the steps of inserting a test nucleic acid fragment into a site of a vector having a promoter, a translational initiation codon downstream of said promoter, a reporter gene which is a structural gene located downstream of said translational initiation codon, which is operably linked to said promoter, which encodes a polypeptide, a fusion polypeptide formed by ligating the N-terminal of said polypeptide to another polypeptide being detectable based on a function of said polypeptide encoded by said reporter gene, said site into which said test nucleic acid fragment is inserted being located downstream of said translational initiation codon and upstream of said reporter gene, said test nucleic acid fragment being one which allows, when inserted, *in-frame* location of said reporter gene with respect to said translational initiation codon when said test nucleic acid is normal type; expressing said test nucleic acid fragment and said reporter gene downstream thereof in the resulting recombinant vector in a host cell, and determining whether said fusion polypeptide having said function of said polypeptide encoded by said reporter gene is produced or not.
2. The method according to claim 1, wherein said translational initiation codon is contained in a second structural gene located upstream of said reporter gene and said test nucleic acid fragment is inserted between said second structural gene and said reporter gene.
3. The method according to claim 1 or 2, wherein said host cell is auxotrophic and said reporter gene is one which converts the auxotroph to prototroph. ~
4. The method according to claim 3, wherein said reporter gene is URA3 gene.
5. The method according to claim 2 or 4, wherein said second structural gene is hemagglutinin gene.
6. The method according to any one of claims 1 to 5, wherein said host cell is a yeast cell.
7. The method according to claim 6, wherein said yeast is *Saccharomyces cerevisiae*.
8. The method according to any one of claims 1 to 7, wherein said test nucleic acid fragment is BRCA1 gene or a fragment thereof.
9. The method according to any one of claims 1 to 7, wherein said test nucleic acid fragment is APC gene or a fragment thereof.
10. The method according to any one of claims 1 to 7, wherein said test nucleic acid fragment is BRCA2 gene or a fragment thereof.
11. The method according to any one of claims 1 to 7, wherein said test nucleic acid fragment is hMSH2 gene or a fragment thereof.

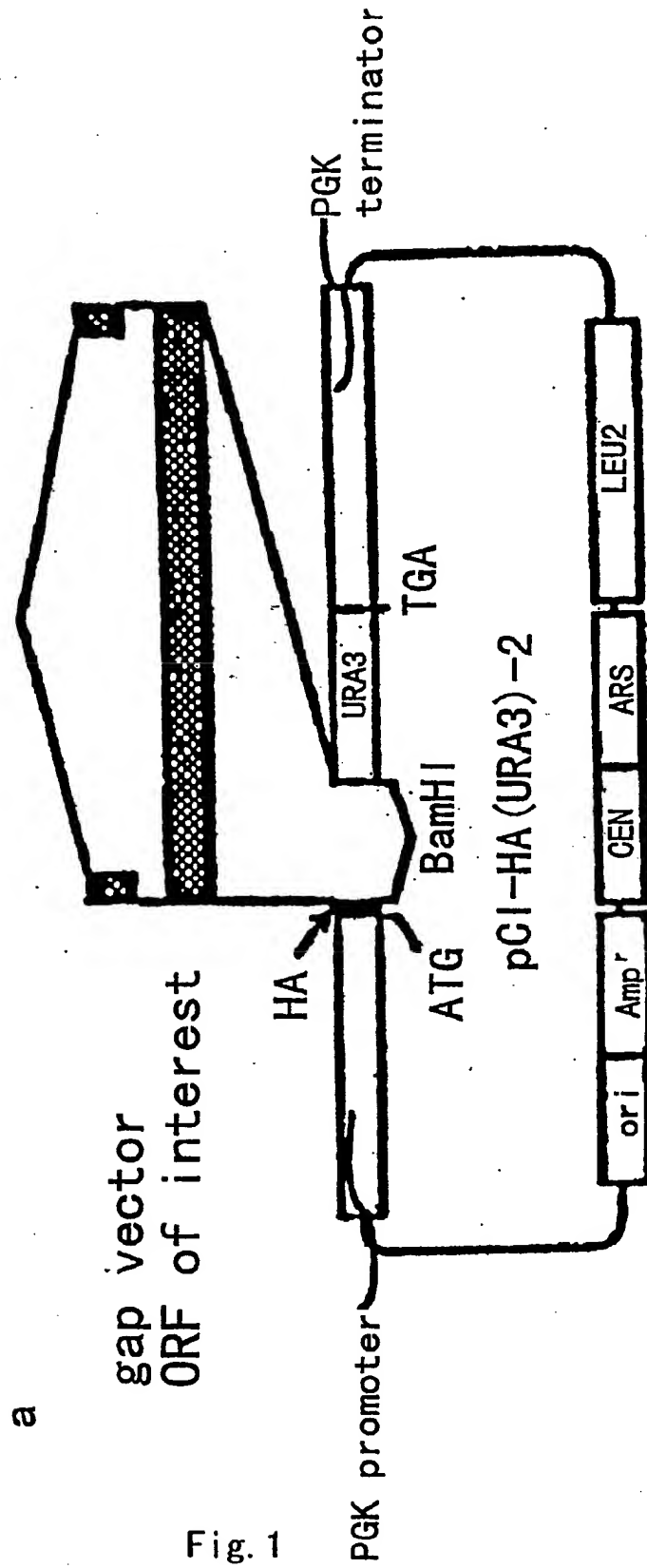


Fig. 1

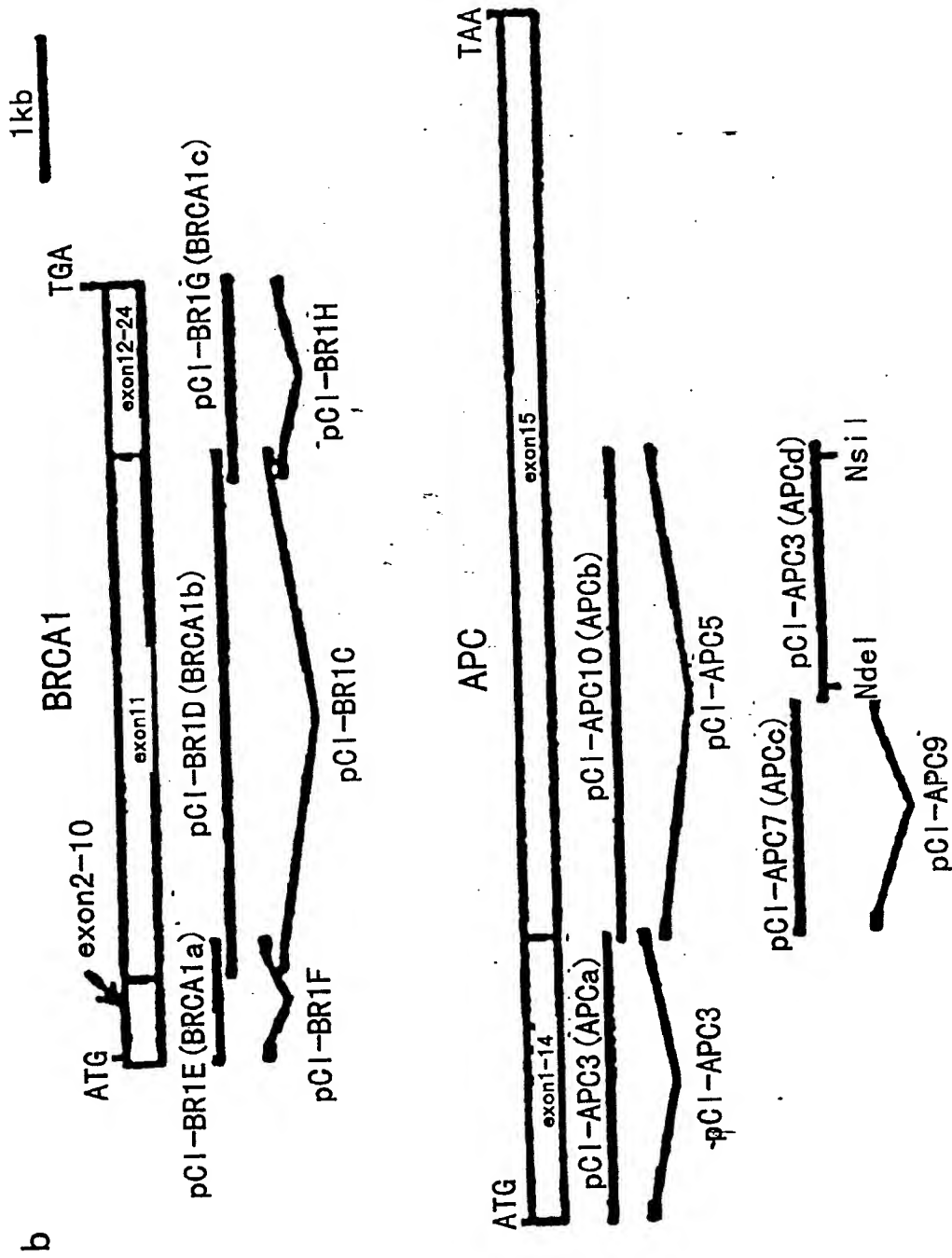


Fig. 1

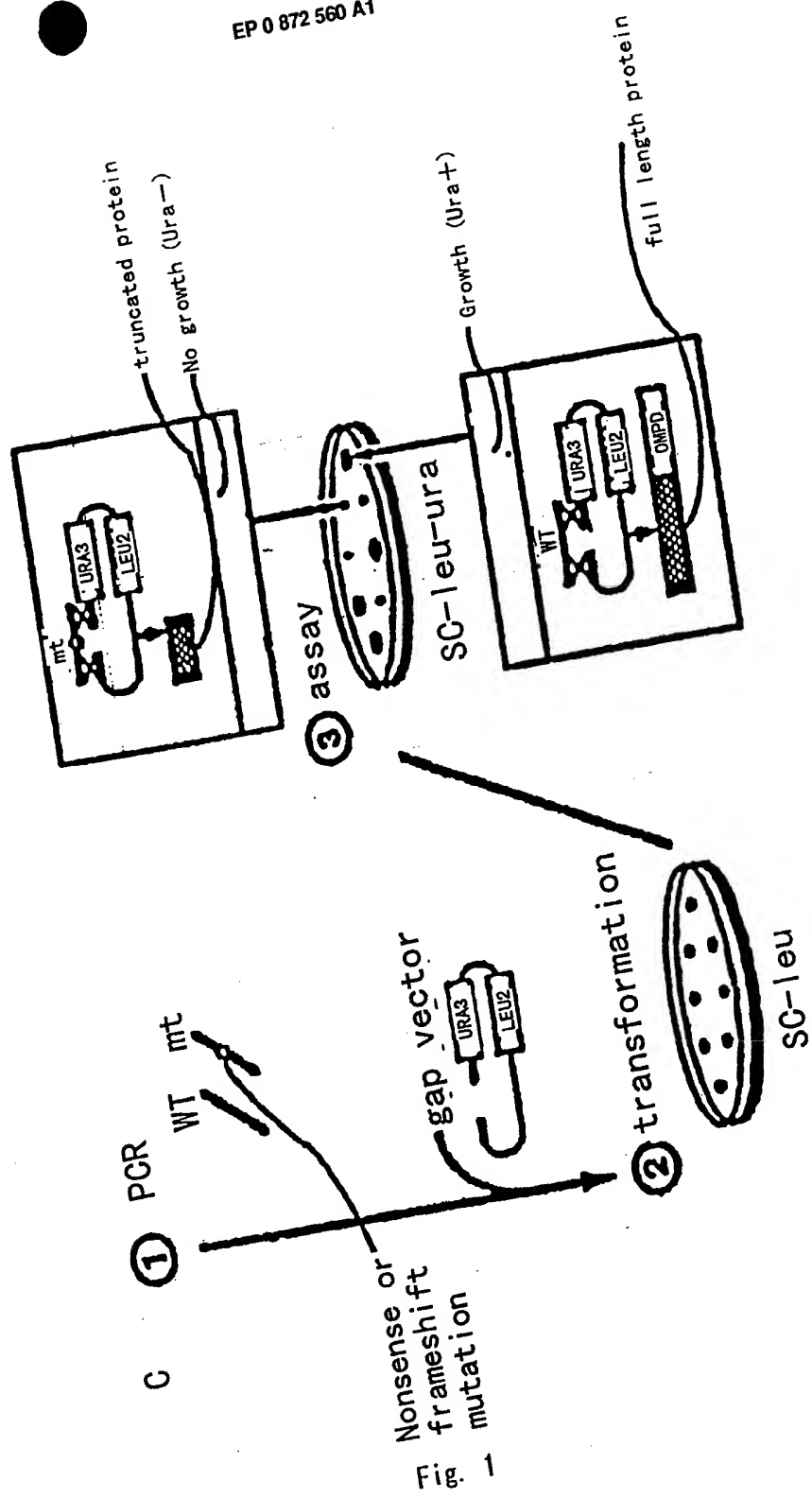


Fig. 1

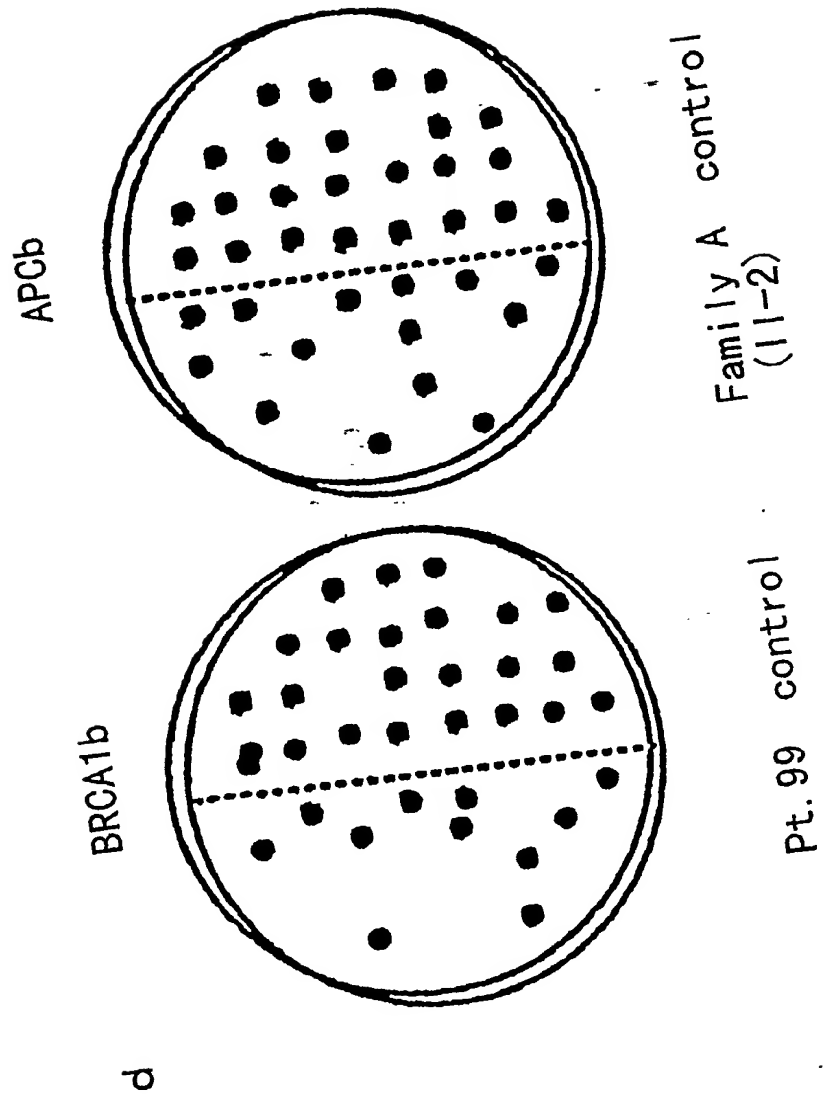


Fig. 1

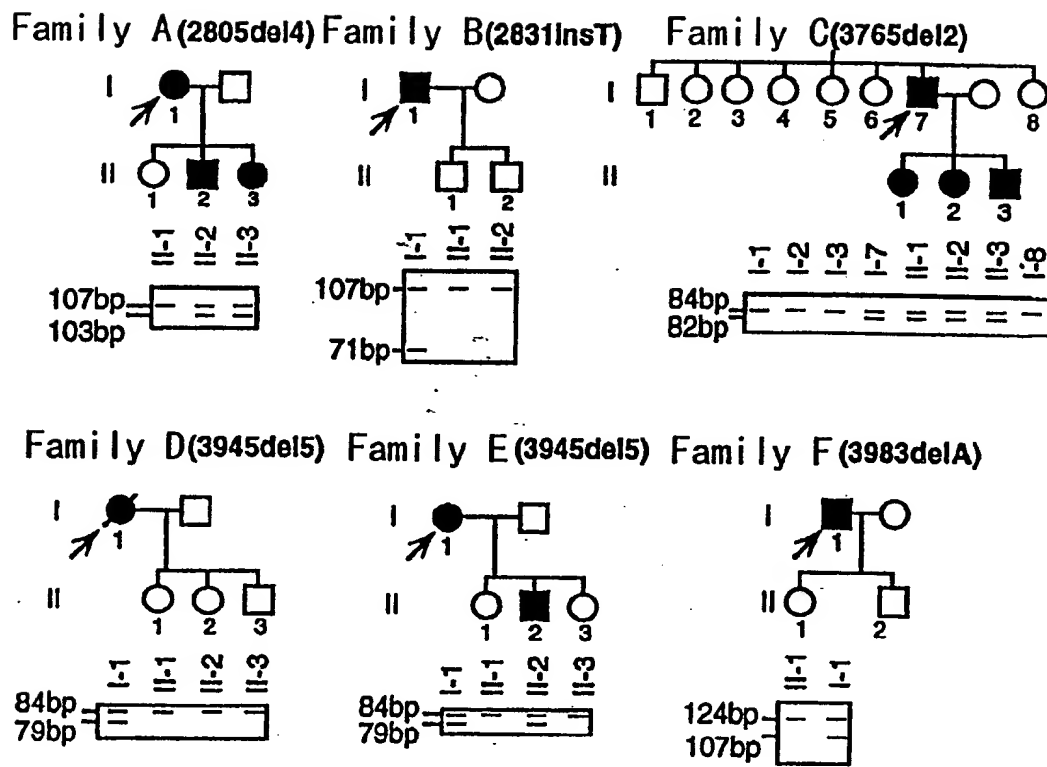


Fig. 2

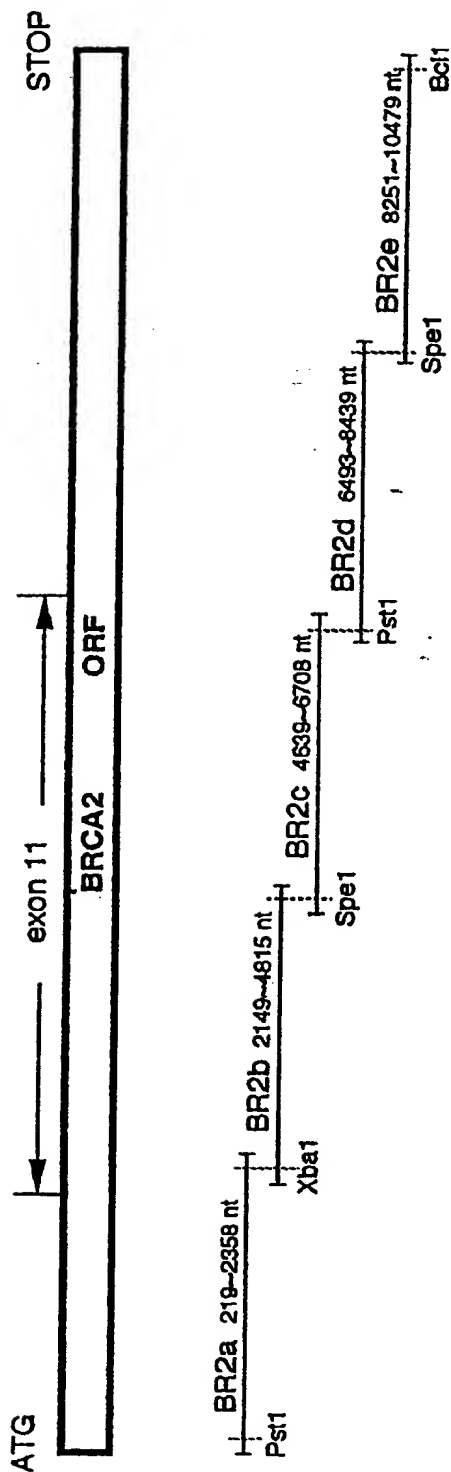


Fig. 3

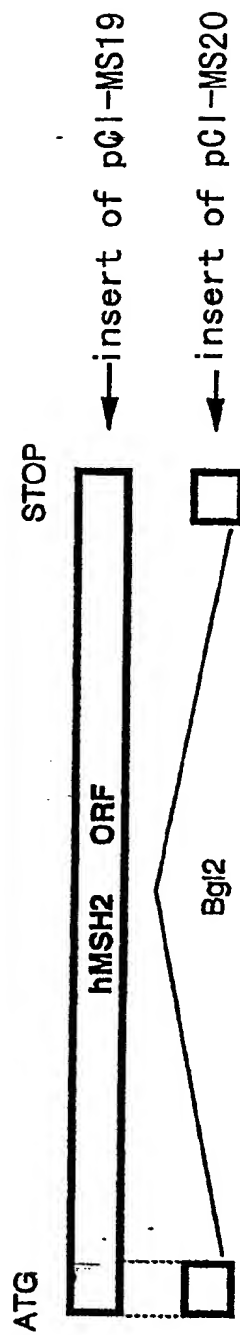


Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/03579

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl ⁶ C12Q1/68 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int. Cl ⁶ C12Q1/02-04, 68 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Medline, Biosis Previews		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Carcinogenesis, Vol. 16, No. 9 (1995), Marwood T.M. et al. "Escherichia coli lacZ strains engineered for detection of frameshift mutations induced by aromatic amines and nitroaromatic compounds" p. 2037-2043	1 - 11
A	Proc. Natl. Acad. Sci. USA., Vol. 86 (1989), Orita M. et al. "Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms" p. 2766-2770	1 - 11
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search December 2, 1997 (02. 12. 97)		Date of mailing of the international search report December 9, 1997 (09. 12. 97)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.		Authorized officer Telephone No.

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